Radiosynthesis and Biological Evaluation of [^{111}In]-5, 10, 15, 20-Tetrakis (Pentafluorophenyl) Porphyrin Complex as a Possible Imaging Agent

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***Abstract*-Porphyrins are interesting derivatives with low toxicity, tumor avidity, and rapid wash-out suggested as potential radiopharmaceuticals in radiolabeled form. In this work, [^{111}In] labeled 5, 10, 15, 20-tetrakis (pentafluorophenyl) porphyrin ([^{111}In]- TFPP) was prepared using freshly prepared [^{111}In]$InCl_3$ and 5,10,15,20-tetrakis (pentafluorophenyl) porphyrin (H_2TFPP) for 60 min at 100°C (radiochemical purity: >99% ITLC, >99% HPLC, specific activity: 13-14 GBq/mmol). Stability of the complex was checked in final formulation and human serum for 48 h. The partition coefficient was calculated for the compound (log P=0.69). The biodistribution of the labeled compound in vital organs of wild-type rats and fibrosarcoma-bearing mice was studied using scarification studies and SPECT up to 24 h. A detailed comparative pharmacokinetic study performed for ^{111}In cation and [^{111}In]-TFPP performed up to 24h. The complex is mostly washed out from the circulation through kidneys and liver. The tumor:blood and tumor: muscle ratios 24 h post injection were 1.44 and 66 respectively.**

Keywords-Porphyrins; In-111; Fibrosarcoma; Biodistribution; SPECT

I. INTRODUCTION

Various metal-porphyrin complexes have shown interesting tumor-avid activity *in vitro* and *in vivo* and have found their ways into clinical studies. Motexafin gadolinium is a novel radiation sensitizer used in the therapy of brain tumors in radiotherapy. BOPP is a boronated porphyrin that with 40 atoms of boron-10 by molecule against one atom alone in the BPA molecule is used in boron-neutron capture therapy. Also tumor accumulation of some indium- porphyrin complexes has already been reported. Radiolabeled porphyrins have been developed for the therapeutic purposes such as, ^{109} Pd-protoporphyrins, ^{109}Pd - porphyrins ^{109} Pd-derivitized porphyrins and ^{188}Re-porphyrins .

Some diagnostic radiolabeled porphyrins have also been reported. For instance, ^{99m}Tc-porphyrin conjugate has evaluated in rodent mammary tumors. Recently ^{99m}Tc-porphine has been developed for imaging despite the high hepatotoxicity . On the other hand the kinetic studies for ^{111}In incorporation into *m*-tetraphenylporphine have been studied while no data was reported for its biological evaluation. We have recently reported the preparation and evaluation of a ^{67}Ga-porphyrin complex as an ultimate ^{68}Ga-PET imaging agent with rapid urinary wash out and fast kinetics.

The interesting physical properties and availability of indium-111 make it an interesting nuclide for radiopharmaceutical research. Due to the interesting pharmacological properties of porphyrins such as solubility in serum, rapid wash-out, tumor avidity and feasible complexation with various bi/tri-valent metals , the idea of developing a possible tumor imaging agent using SPECT (photon emission computed tomography) by incorporating ^{111}In into a suitable porphyrin ligand, *i.e.* H_2TFPP was investigated (Fig. 1).

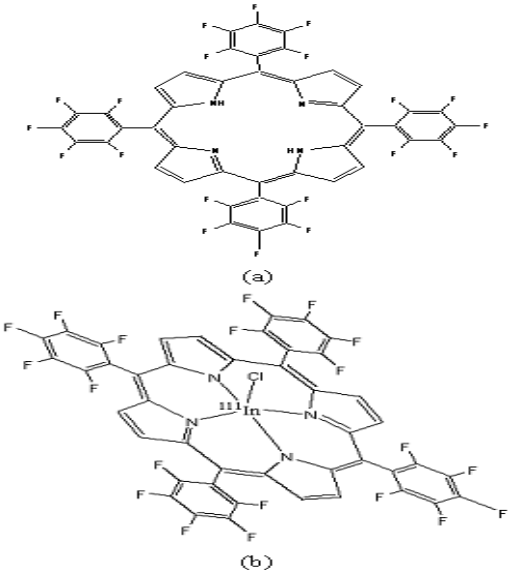

Fig. 1 Structure of H_2TFPP (a) and ^{111}InTFPP (b)

In this work, we report the synthesis, radiolabeling, quality control, stability, partition coefficient determination and biodistribution studies (using SPECT and scarification) of 111In-TFPP in wild-type rats as well as fibrosarcoma-bearing mice. The time/activity diagrams for the labeled compound in vital organs have been plotted compared to indium cation.

II. METHODS

In-111 was produced at the Agricultural, Medical and Industrial Research School (AMIRS), 30 MeV cyclotron (Cyclone-30, IBA) using ^{nat}Cd(p,x)^{111}In reaction. Natural cadmium sulfate with a purity of >99% was obtained from Merck Co. Germany. All chemicals were purchased from Sigma-Aldrich Chemical Co. U.K. Radio-chromatography was performed by Whatman paper using a thin layer chromatography scanner, Bioscan AR2000, Paris, France. Analytical HPLC to determine the specific activity was

performed by a Shimadzu LC-10AT, armed with two detector systems, flow scintillation analyzer (Packard-150 TR) and UV-visible (Shimadzu) using Whatman Partisphere C-18 column 250 × 4.6 mm (Whatman Co. NJ, USA). Calculations were based on the 172 keV peak for ^{111}In. All values were expressed as mean ± standard deviation (Mean± SD) and the data were compared using student T-test. Animal studies were performed in accordance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, second edition.

A. Electroplating of the Natural Cd Targets

In order to prepare Cd targets for the production, cadmium electroplating was performed over a copper surface and was performed according to the previously reported method. Cadmium was electroplated over the copper backing according to the method given in the literature. A mixture of $CdSO_4.8/3H_2O$, KCN, Brij and traces of hydrazine hydrate with a final volume of 450 ml double-distilled water (DDH_2O) at pH=13 was used as the electroplating bath (constant current: 320 mA, stirring rate 780 rpm, time: 0.5 hours). After the deposition of an about 500 mg cadmium layer, the targets were wrapped in Parafilm® coatings to avoid atmospheric oxygen exposure. Finally, the target was sent for irradiation.

B. Production and Quality Control of ^{111}In-$InCl_3$ Solution

Indium-111 chloride was prepared by 22 MeV proton bombardment of the cadmium target at a 30 MeV cyclotron, with a current of 100 μA for 48 min (80 μAh). After the dissolution of the irradiated target by conc. HBr, the solution was passed through a cation exchange dowex 50×8 resin, pre-conditioned by 25 ml of conc. HBr. The resin was then washed by HBr conc. solution (50 ml). In order to remove the undesired impurities of Cd and Cu, the resin was totally washed with DDH_2O. Indium-111 was eluted with 1 N HCl (25 ml) as $^{111}InCl_3$ for labeling use. Gamma spectroscopy of the final sample was carried out counting in an HPGe detector coupled to a Canberra™ multi-channel analyzer for 1000 seconds.

The presence of copper and cadmium impurities in the final solution was checked using acidic dithizone solution and alkaline dimethylglyoxime and NaK tartrate respectively according to the procedure.

C. Quality Control of the Product

Control of Radionuclide purity: Gamma spectroscopy of the final sample was carried out counting in an HPGe detector coupled to a Canberra™ multi-channel analyzer for 1000 seconds.

Chemical purity control: This step was carried out to ensure that the amounts of zinc and copper ions resulting from the target material and backing in the final product are acceptable regarding internationally accepted limits. Chemical purity was checked by differential-pulsed anodic stripping polarography. The detection limit of our system was 0.1 ppm for both zinc and copper ions.

D. Porphyrin derivative (H2TFPP)

The porphyrin H_2TFPP was prepared according to the reported method using freshly distilled pentafluoro benzaldehyde, pyrrole and propionic acid followed by oxidation. UV (CH_2Cl_2) λ_{max} (ε) = 412,506,584 nm. ^{1}HNMR: -2.91 (NH), 9.40 (βH).^{19}FNMR: -136.9 (d), 151.7(t), -161.8 (m),

E. Preparation of $^{[111}$In]-TFPP

The acidic solution (2 ml) of [^{111}In] $InCl_3$ (111 MBq, 3 mCi) was transferred to a 3 ml-borosilicate vial and heated to dryness using a flow of N_2 gas at 50-60°C. Fifty microlitres of TFPP in absolute ethanol (5 mg/ml ≈409 nmoles) was added to the indium-containing vial followed by the addition of acetate buffer pH 5.5 (450 microliteres). The mixture refluxed at 100°C for 60 min. The active solution was checked for radiochemical purity by ITLC and HPLC. The final solution was then passed through a 0.22 μm filter and pH was adjusted to 5.5-7.

F. Quality Control of [^{111}In]-TFPP

Radio thin layer chromatography: A 5 μl sample of the final fraction was spotted on a chromatography Whatman No. 2 paper, and developed in mobile phase mixture, 10% NH_4OAc and methanol 1:1.

High performance liquid chromatography: HPLC was performed with a flow rate of 1 ml/min, pressure: 130 kgF/cm^2 for 20 min. HPLC was performed on the final preparation using a mixture of water:acetonitrile 3:2(v/v) as the eluent by means of reversed phase column Whatman Partisphere C_{18} 4.6 × 250 mm.

G. Determination of Partition Coefficient

Partition coefficient (log *P*) of [^{111}In]-TFPP was calculated followed by the determination of *P* (*P*= the ratio of specific activities of the organic and aqueous phases). A mixture of 1 ml of 1-octanol and 1 ml of isotonic acetate-buffered saline (pH=7) containing approximately 3.7 MBq of the radiolabeled indium complex at 37 ℃ was vortexed 1 min and left 5 min. Following centrifugation at >1200*g* for 5 min, the octanol and aqueous phases were sampled and counted in an automatic well-type counter. A 500 μl sample of the octanol phase from this experiment was shaken again two to three times with fresh buffer samples. The reported log *P* values are the average of the second and third extractions from three to four independent measurements.

H. Stability Tests

The stability of the complex was checked according to the conventional ITLC method. A sample of [^{111}In]-TFPP (37 MBq) was kept at room temperature for 2 days while being checked by ITLC at time intervals in order to check stability in final product using above chromatography system. For serum stability studies, to 36.1 MBq (976 μCi) of [^{111}In]-TFPP was added 500μl of freshly collected human serum and the resulting mixture was incubated at 37 ℃ for 48 h, aliquots (5-μl) were analyzed by ITLC.

I. Induction of Fibrosarcoma Tumors in Mice

Tumor induction performed by the use of poly aromatic hydrocarbon injection in rodents as reported previously. For tumor model preparation, 10μl of 3-methyl cholanthrene solution in extra-virgin olive oil (4 mg/ml) was injected SC to the dorsal area of the mice. After 14-16 weeks the tumor weighed 0.2-0.4 g and was not grossly necrotic. Tumor tissues of some random animals were sent for pathological tests and were diagnosed as fibrosarcoma.

J. Biodistribution in Wild-type Rats and Fibrosarcoma-Bearing Mice

The distribution of the radiolabeled complex among tissues was determined for wild-type rats and bearing

fibrosarcoma-bearing mice immediately after imaging. The total amount of radioactivity injected into each animal was measured by counting the 1-ml syringe before and after injection in a dose calibrator with fixed geometry.

The animals were sacrificed using the animal care protocols at selected times after injection (2 to 24h), the tissues (blood, heart, lung, brain, intestine, feces, skin, stomach, kidneys, liver, muscle and bone) were weighed and rinsed with normal saline and their specific activities were determined with an HPGe detector equipped with a sample holder device as a percent of injected dose per gram of tissues. Blood samples were rapidly taken from the rodent aorta after scarification.

K. Imaging of Wild-Type Rats

Images were taken 2, 4 and 24 hours after administration of the radiopharmaceutical by a dual-head SPECT system. The mouse-to-high energy septa distance was 12 cm. Images were taken from both normal and tumor bearing rats. The useful field of view (UFOV) was 540 mm×400 mm.

III. RESULTS AND DISCUSSION

A. Radio Labeling

Because of the engagement of NH polar functional groups in its structure, labeling of H_2TFPP with indium cation affects its chromatographic properties and the final complex is more lipophilic. Chromatographic system was used for the detection of the radiolabeled compound from the free indium cation. Using 10% NH_4OAc and methanol 1:1 mixture, free indium remains at the origin of the paper as a single peak, while the radiolabeled compound migrates to higher R_f (0.47) (Figure 2).

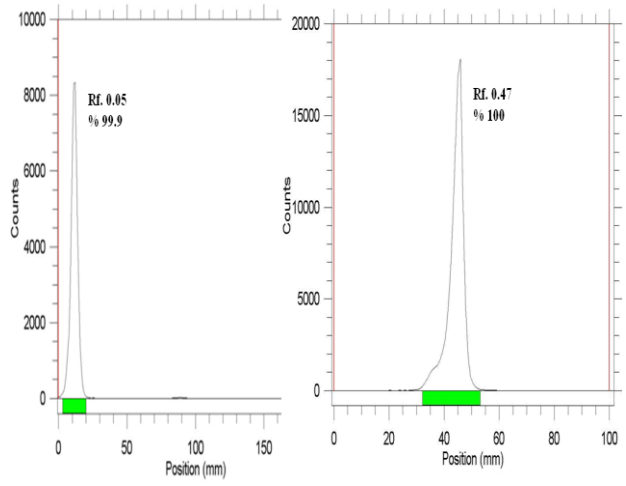

Fig. 2 ITLC of [^{111}In]$InCl_3$ (left) and [111In]InTFPP (right) in a 10% NH4OAc and methanol 1:1 mixture (left) as mobile phase on Whatman No.2 papers

ITLC studies approved the production of a single radiolabeled compound, HPLC studies also demonstrated the existence of only one radiolabeled species using both UV and scintillation detectors. A more fast-eluting compound at 5.29 min (scintillation detector) related to 5.56 min peak (UV detector) demonstrated a more lipophilic compound compared to In cation and unlabeled compound. A second compound eluted at 5.1 min (UV detector) demonstrated non-labeled compound (free ligand and possibly Na-TFPP complex) with a less lipophilic property. Free In-111 cation eluted at 1.22 minutes (not shown) (Figure 3.).

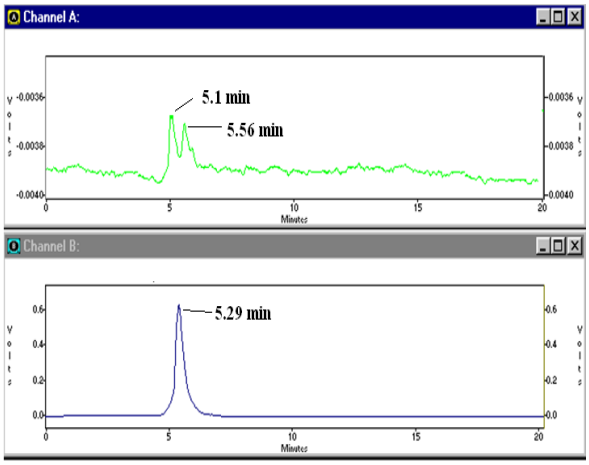

Fig. 3 HPLC chromatograms of [^{111}In]-TPFP on a reversed phase column using acetonitrile:water 40:60, up; UV chromatogram, down; scintillation chromatogram

B. Partition Coefficient of [^{111}In]-TFPP

As expected from the chemical formula in Fig. 1, the lipophilicity of the [^{111}In]-TFPP compound is not rather high due to the ionic nature of the radiocomplex. The measured octanol/water partition coefficient, *P*, for the complex was found to depend on the pH of the solution. At the pH.7 the logP was 0.69. The water solubility of the radiocomplex leads to less unnecessary uptakes in tissues including liver and fat and faster kidney wash-out.

C. Stability

The chemical stability of [^{111}In]-TFPP was high enough to perform further studies. Incubation of [^{111}In]-TFPP in freshly prepared human serum for 2 days at 37 ℃ showed no loss of ^{111}In from the complex. The radiochemical purity of the complex remained at 98% for 2 days under physiologic conditions.

D. Biodistribution in Wild Type Rats

For better comparison biodistribution study was performed for free In^{3+}. The %ID/g data are summarized in Fig. 4. As reported previously, ^{111}In is excreted majorly from gastrointestinal tract (GIT), thus colon and stool activity content are significant while blood stream activity is high at 2-4 h followed by reduction in 24. Bone uptake is also observed after 24 h post injection.

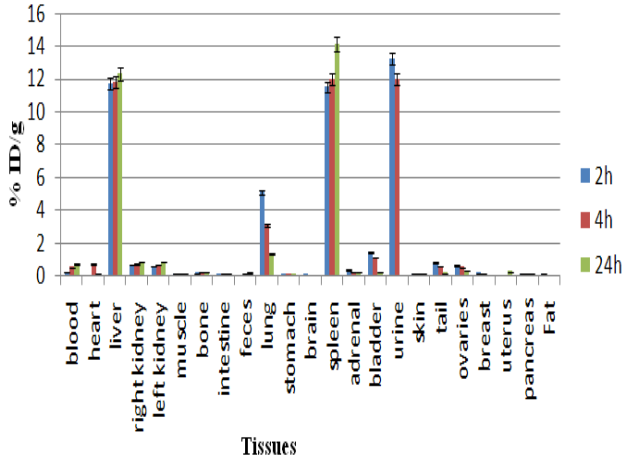

Fig. 4 Biodistribution of [^{111}In] $InCl_3$ (1.85 MBq, 50μCi) in wild-type rats 2, 4 and 24h after injection via tail vein (ID/g%: percentage of injected dose per gram of tissue) (n=5)

As shown in Fig. 4, indium cation almost mimics the ferric cation behavior and is rapidly removed from the circulation and is accumulated in the liver. Also a major fraction is excreted through the kidney as a water soluble cation.

The radiolabeled compound biodistribution is also demonstrated in Fig. 5. Due to the presence of fluorine groups and water solubility of the porphyrin compound as an ionic complex, the major activity in 2 hours post injection is present in lung, liver and spleen, thus the major route of excretion for the labeled compound is through the urinary tract after 24h. Low intestinal activity demonstrates the low hepatobiliary excretion route.

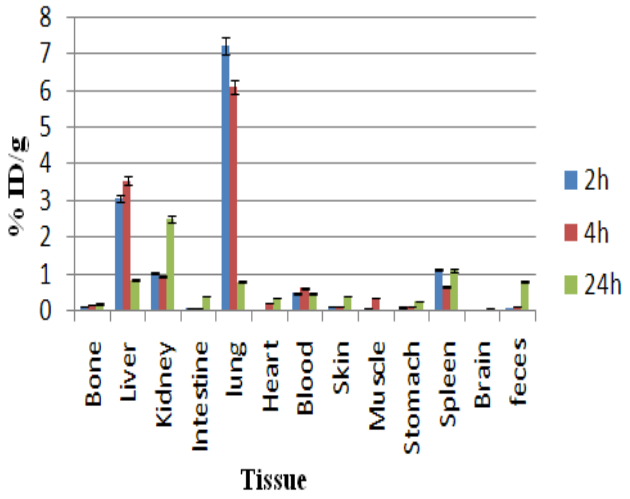

Fig. 5 Biodistribution of [^{111}In]-TFPP (1.85 MBq, 50μCi) in wild type rats 2,4 and 24 h after iv injection via tail vein (ID/g%: percentage of injected dose per gram of tissue calculated based on the area under curve of 172 keV peak in gamma spectrum) (n=3)

Figure 6 demonstrates the comparative study of vital organs uptake for ^{111}In-TFPP and $^{111}InCl_3$ and the kinetic pattern differences for both species. ^{111}In cation is accumulated in the liver in the first 24h post injection slightly, while ^{111}In-TFPP first major excretion route is through the liver.

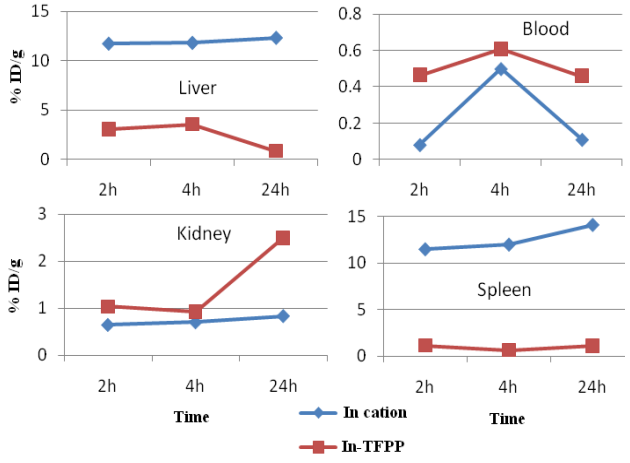

Fig. 6 Comparative blood, bone, kidney, spleen and liver activity for [^{111}In]-TFPP and $^{111}InCl_3$ in wild-type rats 2h, 4h and 24 post injection

Since the urinary tract is a major route of excretion of the porphyrins, and presence of fluorine groups increases the solubility of the ligand in water, the kidney is another excretory organ and shows low activity for the labeled compound esp. after 24h while in the case of In-111 the activity content is almost unchanged until 24h.

No significant difference in blood activity content is observed for free In cation and the labeled compound, since both species are water soluble and excreted from the kidneys and the liver.

Both compounds are excreted through the kidney, however in case of the labeled compound the excretion increases after 24h significantly, while In-111 cation is excreted slowly in 24 h, with an almost steady manner.

As a metal cation In-111 has no spleen uptake while the labeled complex is extremely accumulated in the spleen gradually up to 15%.

E. Biodistribution in Fibrosarcoma-Bearing Mice

Results of the biodistribution studies revealed significant tumor ratio uptake within 24h after injection. The %ID/g data are summarized in Fig. 7.

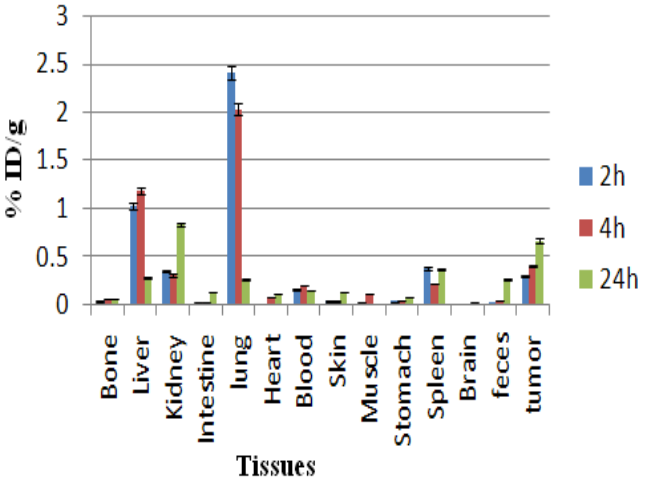

Fig. 7 Biodistribution of [^{111}In]-TFPP (1.85 MBq, 50μCi) in fibrosarcoma-bearing mice 2, 4 and 24 h after iv injection via tail vein (ID/g%: percentage of injected dose per gram of tissue calculated based on the area under curve of 172 keV peak in gamma spectrum) (n=4).

The ratios of tumor to blood and tumor to muscle 2h, 4h and 24h post injection are shown in Table 1.

TABLE 1

Ratio/Time	2h	4h	24h
tumor:blood	0.860381	0.723463	1.456951
tumor:muscle	5.937705	1.253392	66.79999

Since the best target:non-target ratio is observed after 24h for the radio-complex, regarding the tumor:blood and tumor:muscle ratios (1.45 and 66.8 resp.).

F. Imaging of Wild-Type Rats

^{111}In-TFPP imaging in the wild-type rats showed a distinct accumulation of the radiotracer in the chest region all the time after injection. Most of the activity is washed out from the body after 24h and the picture contrast weakened. While a typical In-111 scan is usually high chest and abdomen activity accumulation remaining at least 48 hours in the rat body (Figure 8.).

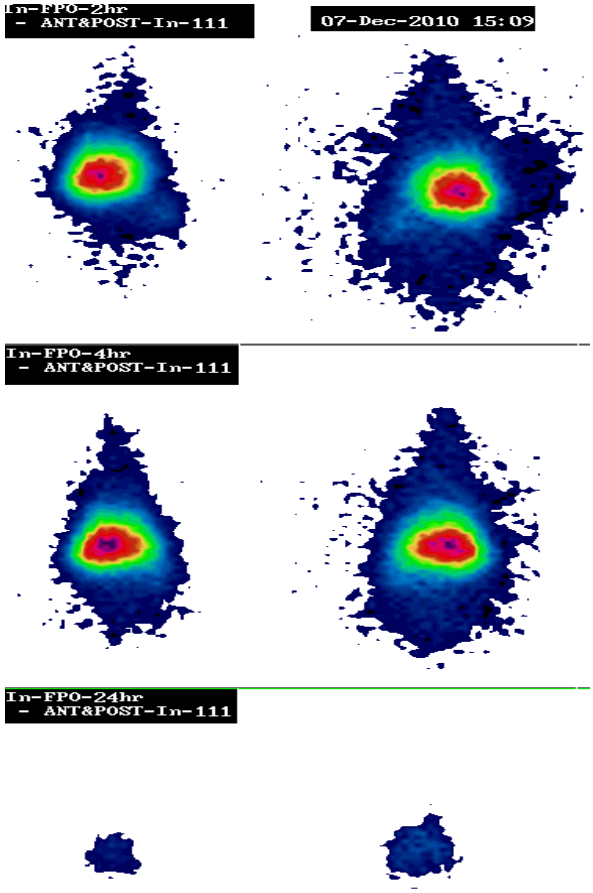

Fig. 8 SPECT images of ^{111}In-TFPP (90 MBq, 22 μCi) in wild-type rats 2h (a), 4h (b) and 24h (c) post injection, (anterior scan; left and posterior scan; right)

IV. CONCLUSION

Total labeling and formulation of [^{111}In]-TFPP took about 30-60 min (RCP >99% ITLC, >99% HPLC, specific activity: 13-14 GBq/mmol). The complex was stable in final formulation and human serum at least for 48 h. At the pH.7, the logP was 0.69. The biodistribution of the labeled compound in vital organs of wild-type rats was studied using scarification studies and SPECT imaging up to 24 h.

A detailed comparative pharmacokinetic study performed for ^{111}In cation and [^{111}In]-TFPP was discussed for 24 h. The complex is mostly washed out from the circulation through kidneys and liver. The tumor:blood and tumor:muscle ratios 24h post injection were 1.45 and 66 respectively. The SPECT images of the radiolabeled compound demonstrated high abdomen uptake 2h-24h post injection which is in agreement with biodistribution data. Higher water solubility of the complex due to ionic nature of the complex is an advantage for rapid wash-out of the complex from the system leading to a enhanced target:non-target ratio. The accumulation of the tracer in other tumor models is under investigation.

REFERENCES

[1] Thomas SR, Khuntia D. Motexafin gadolinium injection for the treatment of brain metastases in patients with non-small cell lung cancer. Int J Nanomed 2007; 2:79–87.

[2] Kahl SB, Koo MS. Synthesis and properties of tetrakiscarborane-carboxylate esters of 2, 4-bis (-dihydroxyethyl) deuteroporphyrin IX. In: Allen BJ, Moore DE, Harrington BV (eds). Progress in Neutron Capture Therapy for Cancer. New York: Plenum Press, (1992) pp. 223-6.

[3] Sasaki K, Yumita N, Nishigaki R, Sakata I, Nakajima S, Umemura S-I, Pharmacokinetic Study of a Indium-porphyrin Photo- and Sono-sensitizer in Tumor-bearing Mice. ATX-70. Jpn J Cancer Res 2001; 92:989–995.

[4] Fawwaz RA, Frye F, Loughman WD, Hemphill W . Survival of skin homografts in dogs injected with ^{109}Pd-protoporphyrin. J Nucl Med 1974; 15:997-1002.

[5] Fawwaz RA, Hemphill W, Winchell HS. Potential use of ^{109}Pd-porphyrin complexes for selective lymphatic ablation. J Nucl Med 1971; 12:231-6.

[6] Chakraborty S, Das T, Banerjee S, Sarma HD, Venkatesh M. Preparation and preliminary biological evaluation of a novel 109Pd labeled porphyrin derivative for possible use in targeted tumor therapy. Q J Nucl Med Mol Imaging 2007; 15:16-23.

[7] Sarma HD, Das T, Banerjee S, Venkatesh M, Vidyasagar PB, Mishra KP. Biologic evaluation of a novel ^{188}Re-labeled porphyrin in mice tumor model. Cancer Biother Radiopharm 2010; 25:47-54.

[8] Murugesan S, Shettyc SJ, Srivastava TS, Noronha OPD, Samuel AM. A technetium-99m-labelled cyclam acid porphyrin (CAP) for tumour imaging. Applied Radiat Isotopes 2001; 55:641-646.

[9] Wang A-Y, Lin J-L, Lin W-C. Studies on the porphine labeled with ^{99m}Tc–pertechnetate. J Radioanal Nucl Chem 2010; 284:121-28.

[10] Nunn AD. Medical Radionuclides and the Quality of Radiopharmaceuticals, The kinetics of incorporation of ^{111}In into m-tetraphenylporphine, J Radioanal Nucl Chem 1978l; 53:291-298.

[11] Fazaeli Y, Jalilian AR, Amini M. M, Rahiminejad-kisomi A, Rajabifar S. Bolourinovin F, Moradkhani S. Preparation and preliminary evaluation of [^{67}Ga]-tetra phenyl porphyrin complexes as possible imaging agents J Radioanal Nucl Chem 2011; 288:17-24.

[12] Firestone RB, Shirley VS, Baglin CM, Zipkin J, In: Table of isotopes. 8th edition, John Wiley and Sons, p.1447, New York, (1996).

[13] Falk JE, Porohyrins and metalloporphyrins. New York, Elsevier Science Publishing 1975.

[14] Sadeghpour H, Jalilian AR, Akhlaghi M, Kamali-dehghan M, Mirzaii M. Preparation and Biodistribution of [^{111}In]-rHuEpo for Erythropoietin Receptor Imaging. J Radioanal Nucl Chem. 2008; 278:117-122.

[15] Mirzaii M, Afarideh H, Haji-Saied SM, Ardaneh K. Production of ^{111}In by irradiation of natural cadmium with deuterons and protons in NRCAM cyclotron. In: Proc on 15th Int conf on cyclotrons and their applications. Caen, France, 65-67 (1998).

[16] Marczenko Z. In: Spectrophotometric determination of elements. New York: John Wiley & Sons, 1976:238-240.

[17] Marczenko Z. In: Spectrophotometric determination of elements. New York: John Wiley & Sons, 1976:176-179.

[18] Adler AD, Longo FR, Finarelli JD, Goldmacher J, Assour J, Korsakoff L. A simplified synthesis for meso-tetraphenylporphine. J Org Chem 1967; 32: 476.

[19] Jalilian AR, Rowshanfarzad P, Sabet M, Novinrooz A, Raisali G. Preparation of [^{66}Ga]Bleomycin Complex as a Possible PET Radiopharmaceutical. J Radioanal Nucl Chem 2005; 264: 617.

[20] DiGiovanni J, Rymer J, Slaga TJ, Boutwell RK. Anticarcinogenic and co-carcinogenic effects of benzo[e]pyrene and dibenz [a,c]anthracene on skin tumor initiation by polycyclic hydrocarbons. Carcinogenesis 1982; 3(4):371-5.